Discussion

Dr Y. Joseph Woo *(Philadelphia, Pa).* You should be congratulated for a fantastic study, particularly with the technical aspects that you showed and also the creative water bath assay. You answered a lot of the potential questions that the audience would have with in your last two slides.

Your study is very good at showing the effects of your therapy. To take your study to the next level, you should show some mechanistic components. The investigators who are doing a lot of the stem cell transplantation work are thinking that there is not much cross-linking of the cells and probably not a tremendous contractile contribution from stem cell transplantation, but rather some sort of biofactory effect. You did measure a multitude of different parameters. Can you comment on some of the other things that you may be in the process of measuring that would perhaps better elucidate the mechanism in effect here?

Dr Molina. Thank you, Dr Woo, for your comments and questions. Regarding the mechanisms of action of bone marrow–derived MSCs, I think that is one of the fundamental questions in this area of research, as you mentioned. The contribution of MSCs to the improvement in contractile function of these hearts is probably mild. Contractile function is improved in treated animals in comparison with the control group at 21 and 28 days after therapy, but if we compare treated animals with baseline contractility before cell transplantation, it is probably similar. We think that both systolic and diastolic function became stable after treatment. Treatment with bone marrow–derived MSCs, at least in this study, avoided the deterioration in contractility that occurs with progression of heart failure. Whether the cells really differentiate into cardiomyocytes is still unknown. We are still running studies to identify these cells at later intervals, and we are planning to do it with confocal microscopy. However, there are other findings that I did not present that are also significant in terms of alternative potential mechanisms of action. We have measured tissue growth factors in these hearts, and several of them are significantly increased in the cell treatment group: for example, insulin-like growth factor, hepatocyte growth factor, and platelet-derived growth factor. We think that the paracrine effects that these cells exert on native cardiomyocytes might play a very significant role in terms of the mechanisms of action.

Dr Beat H. Walpoth *(Geneva, Switzerland).* *Although* you stated improvements in remodeling, function, and anti-inflammation, so far as I have seen, you have only shown one slide with a few labeled cells at a very early time point after injection. Did you find any surviving cells at 28 days and did you try to quantify them? Additionally, differentiation and characterization of the injected cells would be helpful to support your hemodynamic results.

Dr Molina. Thank you for your question. These cells were marked not only with BrdU, as I showed in two slides, but also with green fluorescent protein (GFP). Both of these markers tend to disappear with cellular division. By Western blot analysis we were able to find cells positive for GFP only up to 14 days after cell injection. We did not find any GFP after that time interval. As I mentioned before, we are still in the process of obtaining images with confocal microscopy at later time intervals, which represents a challenging task.

In our next studies we are planning to use GFP with a lentiviral GFP adenovirus transfection, which is a much more stable transfection and is going to allow us to trace these cells for longer periods of time.

Dr Mark J. Krasna *(Baltimore, Md).* Dr Molina, I have one quick question. Technically, you were able to do two sternotomies and a swim time. Did any of these rats die? In the manuscript it appears that they all survived.

Dr Molina. Not all of them survived. In the first operation, aortic clip placement, there was a survival of about 70%. About 15% of the animals died during or right after the operation, usually of respiratory complications, and another 15% usually died during the follow-up period as heart failure developed.